

FILE 'REGISTRY' ENTERED AT 10:48:05 ON 30 SEP 2005

=> S RESTRICTION ENZYME/CN  
L1 1 RESTRICTION ENZYME/CN

FILE 'CAPLUS' ENTERED AT 10:48:18 ON 30 SEP 2005

=> S RESTRICTION(W) (ENZYME OR ENDONUCLEASE); S L1; S L2, L3  
98266 RESTRICTION  
12900 RESTRICTIONS  
110352 RESTRICTION  
(RESTRICTION OR RESTRICTIONS)  
750614 ENZYME  
433967 ENZYMES  
948402 ENZYME  
(ENZYME OR ENZYMES)  
26959 ENDONUCLEASE  
8081 ENDONUCLEASES  
31291 ENDONUCLEASE  
(ENDONUCLEASE OR ENDONUCLEASES)  
L2 32039 RESTRICTION(W) (ENZYME OR ENDONUCLEASE)

L3 3916 L1

L4 32352 (L2 OR L3)

=> S CORYNEBACTERIUM  
11598 CORYNEBACTERIUM  
638 CORYNEBACTERIA  
L5 11771 CORYNEBACTERIUM  
(CORYNEBACTERIUM OR CORYNEBACTERIA)

=> S CSTMI OR (CSTM(W) (I OR 1))  
1 CSTMI  
9 CSTM  
4118830 I  
8411026 1  
0 CSTM(W) (I OR 1)  
L6 1 CSTMI OR (CSTM(W) (I OR 1))

=> D CBIB ABS

L6 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2005 ACS on STN  
2005:36466 Document No. 142:109453 Novel type II restriction endonuclease,  
CstMI, obtainable from corynebacterium striatum m82b. Morgan,  
Richard D.; Bhatia, Tanya (USA). U.S. Pat. Appl. Publ. US 2005009034 A1  
20050113, 28 pp. (English). CODEN: USXXCO. APPLICATION: US 2003-616689  
20030710.

AB In accordance with the present invention, there is provided a novel type II  
restriction endonuclease, obtainable from Corynebacterium striatum M82B,  
hereinafter referred to as "CstMI", which endonuclease recognizes the nucleotide  
sequence 5'-AAGGAG-3' in a double-stranded DNA mol. as shown below, 5'-  
AAGGAGN20↓-3' and 3'-TTCTCN18↑-5', (wherein G represents guanine, C represents  
cytosine, A represents adenine, T represents thymine and N represents either G,  
C, A, or T). The new CstMI cleaves sequence at the phosphodiester bonds between  
the 20th and the 21th nucleotides 3' to the recognition sequence in the 5'-  
AAGGAG-3 strand of the DNA, and between the 18th and 19th nucleotides 5' to the  
recognition sequence in the complement stand, 5'-CTCCTT-3', to produce a 2 base  
3' extension. The new CstMI also possesses a second enzymic activity that  
recognizes the same DNA sequence, 5'-AAGGAG-3', but modifies this sequence by the  
addition of a Me group to prevent cleavage by the CstMI endonuclease activity.

=> S L4 AND L5  
L7 111 L4 AND L5

=> S L4(6A)L5  
L8 16 L4(6A)L5

=> S L8 NOT L6  
L9 15 L8 NOT L6

=> D 1-15 CBIB ABS

L9 ANSWER 1 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

2004:65494 Document No. 140:247774 Identification of *Corynebacterium bovis* by endonuclease restriction analysis of the 16S rRNA gene sequence. Huxley, J. N.; Helps, C. R.; Bradley, A. J. (Department of Clinical Veterinary Science, University of Bristol, Bristol, BS40 5DU, UK). Journal of Dairy Science, 87(1), 38-45 (English) 2004. CODEN: JDSCAE. ISSN: 0022-0302. Publisher: American Dairy Science Association.

AB Despite its high prevalence within the bovine mammary gland, *Corynebacterium bovis* is considered a minor pathogen and of limited clin. significance. It has been suggested that intramammary infection with *C. bovis* may protect quarters against subsequent infection with other pathogens. The literature has produced much conflicting data on the subject. A possible explanation for some of the divergence of opinion on the subject is incorrect identification of isolates in previous studies. This paper describes a novel method for differentiating *C. bovis* from other lipophilic *Corynebacterium* species based on endonuclease restriction anal. The 16S rRNA gene sequences for all known lipophilic *Corynebacterium* species were obtained from published data and analyzed. It was predicted that endonuclease restriction with HindIII and SmaI could be used to differentiate *C. bovis* from all other known lipophilic *Corynebacterium* species. The method was successfully employed to identify 741 of 762 (97.2%) lipophilic *Corynebacterium* species as *C. bovis*. Twenty one (2.8%) were identified as species other than *C. bovis*. Using this technique, it was demonstrated that it is not safe to assume that all lipophilic coryneform organisms isolated from bovine milk samples are *C. bovis*. This method is an alternative to more traditional methods of identification in large scale studies until methods such as 16S rRNA gene sequencing become more widely available.

L9 ANSWER 2 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

2003:789879 Document No. 139:376166 Method of differentiation of *Corynebacterium diphtheriae* comprising functionally active diphtheria toxin gene from *corynebacterium diphtheriae* comprising functionally inactive diphtheria toxin gene. Mel'nikov, V. G.; Mazurova, I. K.; Kombarova, S. Yu. (Gosudarstvennoe Uchrezhdenie Moskovskii Nauchno-Issledovatel'skii Institut Epidemiologii i Mikrobiologii im. G. N. Gabrichevskogo, Russia). Russ. RU 2209831 C1 20030810, No pp. given (Russian). CODEN: RUXXE7. APPLICATION: RU 2001-135409 20011228.

AB The invention relates to a method of differentiation of *Corynebacterium diphtheriae* comprising functionally active diphtheria toxin gene from *Corynebacterium diphtheriae* comprising functionally inactive diphtheria toxin gene. The method involves isolation of DNA from the strain *C. diphtheriae* or from clinic material, carrying out polymerase chain reaction with simultaneous incorporation of primers 5'-GTCAAAGTGACGTATCCAGGA-3' and 5'-CACGGGTTTCAAATTAATCTC-3', preparing amplicons of diphtheria toxin gene fragment, restriction of these amplicons with restriction endonuclease Bsm FI followed by electrophoresis of restriction fragments in agarose gel. Then differentiation is carried out by comparison of diphtheria toxin gene amplicons in analyzed sample with two control samples containing amplicons of functionally active and inactive

diphtheria toxin gene. The invention provides a rapid and efficient method of differentiation of *Corynebacterium diphtheriae*.

L9 ANSWER 3 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

2000:888618 Document No. 134:97061 Isolation-purification of a new restriction endonuclease, CgII and its optimum reaction condition. Ra, Sung Ryong; Ri, Dong Chan (S. Korea). Choson Minjujuui Inmin Konghwaguk Kwahagwon Tongbo (4), 36-38 (Korean) 2000. CODEN: CKWTAN. ISSN: 0366-6662. Publisher: Science and Technological Publishing House.

AB A new restriction endonuclease CgII is isolated and purified from *Corynebacterium glutamicum* 165 by a ultrasonication, Biogel filtered phosphocellulose and DEAE-cellulose column chromatog. We establish some reasonable reaction conditions for the purified restriction endonuclease, CgII.

L9 ANSWER 4 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

2000:465779 Document No. 133:54518 Gene and expression of mannose enzyme from *Corynebacterium glutamicum*. Lee, Jung-ki; Sung, Moon-hee; Yun, Ki-hong; Oh, Tae-kwang (Kist, S. Korea). Repub. Korea KR 9608410 B1 19960626, No pp. given (Korean). CODEN: KRXXFC. APPLICATION: KR 1993-3655 19930301.

AB Mannose enzyme II gene is prepared by : separating chromosome DNA from *Corynebacterium glutamicum* KCTC 1445; partly cutting it with restriction enzymes; obtaining gene library of the *Corynebacterium glutamicum* by adopting *E. coli* as a host; and cloning into plasmid pCTS3 (KCTC 85729) among the library. It is useful for amino acid fermentation

L9 ANSWER 5 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1998:241527 Document No. 129:24868 Isolation and characterization of CspBI, a novel NotI isoschizomer from *Corynebacterium* species B recognizing 5'-GC↓GGCCGC-3'. Sokolov, Nikolai N.; Eldarov, Michael A.; Rina, Mary; Korolev, Sergey V.; Markaki, Maria; Kalugin, Alexei A.; Omelyanuk, Natalia M.; Skryabin, Konstantin G.; Bouriotis, Vassilis (Institute of Biomedical Chemistry RAMS, Moscow, 119832, Russia). Biochemistry and Molecular Biology International, 44(3), 433-441 (English) 1998. CODEN: BMBIES. ISSN: 1039-9712. Publisher: Academic Press.

AB Sixty-seven bacterial strains were surveyed for the presence of type II restriction endonucleases, especially concerning super-rare-cutting enzymes. Fourteen strains were found to contain specific enzymes. One of them, restriction endonuclease CspBI from *Corynebacterium* species B, was purified and characterized as an isoschizomer of endonuclease NotI, which recognizes the palindromic octanucleotide sequence 5'-GC↓GGCCGC-3' and cleaves at the position shown by the arrow. A comparison between the cleavage patterns on different DNAs, obtained with partially purified endonucleases from other detected producents, including some strains of *Corynebacterium*, *Cellulomonas*, and *Rhizobium*, showed that these enzymes do not belong to super-rare-cutting restriction endonucleases.

L9 ANSWER 6 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1998:63370 Document No. 128:125154 Restrictive endonuclease CgII from *Corynebacterium glutamicum*. Ra, Sung Ryong (S. Korea). Choson Minjujuui Inmin Konghwaguk Kwahagwon Tongbo (3), 41-43 (Korean) 1997. CODEN: CKWTAN. ISSN: 0366-6662. Publisher: Science and Technological Publishing House.

AB A new type-II restrictive endonuclease designated CgLI was identified from *Corynebacterium glutamicum* 165. This endonucleases cleaved bacteriophage  $\lambda$  DNA at nine sites and plasmid pBR322 DNA at one site.

L9 ANSWER 7 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

- 1997:625197 Document No. 127:303866 Cloning and characterization of the genes of the CeqI restriction-modification system. Izsvak, Zsuzsa; Jobbagy, Zsolt; Takacs, Imre; Duda, Erno (Biological Research Centre, Institute of Biochemistry, Hungarian Academy of Sciences, Szeged, H-6701, Hung.). International Journal of Biochemistry & Cell Biology, 29(6), 895-900 (English) 1997. CODEN: IJBBFU. ISSN: 1357-2725. Publisher: Elsevier.
- AB Two genes from *Corynebacterium equi*, a Gram-pos. bacterium producing the CeqI restriction-modification enzymes were cloned and sequenced. In vivo restriction expts., DNA and amino acid sequence data suggest that the two genes code for the endonuclease and the methyltransferase enzymes. However, when the two genes are expressed in *E. coli*, practically no enzyme activity can be detected in the supernatants of sonicated cells. Based on the DNA sequence data CeqI restriction endonuclease (an EcoRV isoschizomer) consists of 270 amino acid residues with a predicted mol. mass of 31.6 kDa, in good agreement with the previously measured 32±2 kDa. The methyltransferase is 517 residues long (approx. 60 kDa). The two genes are in opposite orientation and overlap by 37 base pairs on the chromosome. The deduced amino acid sequence of the putative endonuclease gene revealed long stretches of hydrophobic amino acids, that may form the structural basis of the unusual aggregation properties of the restriction endonuclease. The amino acid sequence of the methylase shows homologies with other type II methyltransferases.
- L9 ANSWER 8 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
- 1995:602056 Document No. 123:5056 Molecular epidemiology of *Corynebacterium diphtheriae* from northwestern Russia and surrounding countries studied by using ribotyping and pulsed-field gel electrophoresis. Zoysa, Aruni De; Efstratiou, Androulla; George, R. C.; Jahkola, M.; Vuopio-Varkila, Jaana; Deshevoi, S.; Tseneva, Galina; Rikushin, Y. (Respiratory and Systemic Infection Laboratory, Central Public Health Laboratory, London, NW9 5HT, UK). Journal of Clinical Microbiology, 33(5), 1080-3 (English) 1995. CODEN: JCMIDW. ISSN: 0095-1137.
- AB A selection of 100 *Corynebacterium diphtheriae* isolates from asymptomatic carriers and clin. cases from five regions in northwestern Russia were examined. Six addnl. isolates from patients in Finland and Estonia with epidemiol. links to Russia were also examined. All isolates were characterized by biotyping, toxigenicity testing, ribotyping, and pulsed-field gel electrophoresis (PFGE). Hybridization of genomic DNA digested with BstEII revealed five ribotype patterns among the biotype gravis isolates (G1 through G5) and two patterns among the biotype mitis isolates (M1 and M2). PFGE using SfiI was not able to distinguish between ribotypes G1, G2, and G4. The predominant ribotype pattern, G1, found in cases of disease in all the areas studied, appears to be disseminating, in view of the isolates received from imported cases in Finland and Estonia. Among the 106 isolates examined, 68 produced pattern G1 and 24 produced pattern M1. Most of the M1 isolates were from the Leningrad Oblast region. Distinct ribotypes such as G2, G3, G4, G5, and M2 could represent endemic disease.
- L9 ANSWER 9 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN
- 1995:233153 Document No. 122:98439 Cloning and characterization of a DNA region encoding a stress-sensitive restriction system from *Corynebacterium glutamicum* ATCC 13032 and analysis of its role in intergeneric conjugation with *Escherichia coli*. Schaefer, Andreas; Schwarzer, Astrid; Kalinowski, Joern; Puehler, Alfred (Department of Genetics, University of Bielefeld, Bielefeld, 33501, Germany). Journal of Bacteriology, 176(23), 7309-19 (English) 1994. CODEN: JOBAAY. ISSN: 0021-9193. Publisher: American Society for Microbiology.
- AB RP4-mediated transfer of mobilizable plasmids in intergeneric conjugation of *Escherichia coli* donors with *Corynebacterium glutamicum* ATCC 13032 is severely affected by a restriction system in the recipient that can be inactivated by a variety of exogenous stress factors. In this study a rapid test procedure based on intergeneric conjugal plasmid transfer that permitted the distinction between restriction-neg. and restriction-pos. *C. glutamicum* clones was developed. By

using this procedure, clones of the restriction-deficient mutant strain *C. glutamicum* RM3 harboring a plasmid library of the wild-type chromosome were checked for their restriction properties. A complemented clone with a restriction-pos. phenotype was isolated and found to contain a plasmid with a 7-kb insertion originating from the wild-type chromosome. This plasmid, termed pRES806, is able to complement the restriction-deficient phenotype of different *C. glutamicum* mutants. Sequence anal. revealed the presence of two open reading frames (orf1 and orf2) on the complementing DNA fragment. The region comprising orf1 and orf2 displayed a strikingly low G+C content and was present exclusively in *C. glutamicum* strains. Gene disruption expts. with the wild type proved that orf1 is essential for complementation, but inactivation of orf2 also resulted in a small but significant increase in fertility. These results were confirmed by infection assays with the bacteriophage CL31 from *Corynebacterium lilium* ATCC 15990.

L9 ANSWER 10 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1993:163797 Document No. 118:163797 Purification and characterization of CeqI restriction endonuclease. Izsvak, Zsuzsa; Jobbagy, Zsolt; Duda, Erno (Inst. Biochem., MTA Biol. Res. Cent., Szeged, H-6701, Hung.). Zeitschrift fuer Naturforschung, C: Journal of Biosciences, 47(11-12), 830-4 (English) 1992. CODEN: ZNCBDA. ISSN: 0341-0382.

AB CeqI, a type II restriction endonuclease and an isoschizomer of EcoRV, was purified to apparent homogeneity by a combination of salt precipitation, ion-exchange, dye-affinity, and hydrophobic interaction chromatogs. The crude enzyme was present in the form of large aggregates that could be pelleted by high-speed centrifugation. The enzyme was not associated with cellular membranes, although nonionic detergents lowered the apparent size of the aggregates. The purified enzyme also showed a tendency to form large-mol.-weight (66-600 kDa) complexes under physiol. conditions, in the absence of cleavable DNA. The enzyme formed smaller complexes in the presence of DNA and nonionic detergents and dissociated into subunits (and underwent reversible loss of activity) in the presence of high concns. of salts. According to SDS-gel electrophoresis and sedimentation anal., the mol. weight of the monomer was 32 kDa. The enzyme had a rather broad pH optimum, extending into the alkaline range and lost specificity and activity in buffers below pH 6.

L9 ANSWER 11 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1989:150362 Document No. 110:150362 'Star' activity and complete loss of specificity of CeqI endonuclease. Izsvak, Zsuzsa; Duda, E. (Inst. Biochem., Hung. Acad. Sci., Szeged, H-6701, Hung.). Biochemical Journal, 258(1), 301-3 (English) 1989. CODEN: BIJOAK. ISSN: 0306-3275.

AB Restriction endonuclease CeqI of *Corynebacterium equii*, an isoschizomer of EcoRV, exhibits EcoRV\* activity, a relaxation of specificity in the presence of Mn<sup>2+</sup>, DMSO, or glycerol. The enzyme cleaves a set of sequences that differ from the canonical GATATC by only 1 nucleotide in positions 2, 3, 4, or 5. Two of these sequences are not cleaved if modified by dam methylase. A further loss of specificity can be observed in circumstances less favorable for the enzyme, namely low-ionic-strength buffers of pH values <6.0 or >9.4. This activity seems to cleave DNA at any sequence, producing a smear instead of well-defined bands. Partial renaturation of the denatured enzyme gives rise to a similar nonspecific nuclease activity.

L9 ANSWER 12 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1989:55988 Document No. 110:55988 Fermentative production of restriction endonuclease Ceq I. Duda, Erno; Fejes, Maria; Orosz, Andras; Izsvak, Zsuzsa (Magyar Tudományos Akademia, Szegedi Biológiai Központja, Hung.). Hung. Teljes HU 45093 A2 19880530, 13 pp. (Hungarian). CODEN: HUXXB. APPLICATION: HU 1986-4762 19861118.

AB The title process is carried out with *Corynebacterium equii* OK 00355. The enzyme, which has a restriction site at GAT-ATC, is extracted at the late log or

stationary phase of the culture. The bacterial mass was ultrasonicated at pH 7.4 (tris-HCl buffer), followed by centrifuging and treatment of the supernatant with streptomycin sulfate. Following centrifuge, the supernatant was 70% saturated with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, centrifuged again and the precipitate dissolved in the pH 7.4 buffer. The solution was dialyzed against the same buffer, followed by column chromatog. on DEAE cellulose. The column was equilibrated with 10 mM HSCH<sub>2</sub>CH<sub>2</sub>OH. Elution with 200 mM KCl gave the enzyme in high purity.

L9 ANSWER 13 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1987:591853 Document No. 107:191853 Restriction endonuclease map of plasmid pXZ10145 in *Corynebacterium*

glutamicum and the construction of composite plasmid. Zheng, Zhaoxin; Ma, Chuping; Yan, Weiyao; He, Peifu; Mao, Yixiang; Sun, Wei; Lei, Zaozu; Zhu, Ping; Wu, Jufen (Inst. Genet., Fudan Univ., Shanghai, Peop. Rep. China). Shengwu Gongcheng Xuebao, 3(3), 183-8, 1 plate (Chinese) 1987. CODEN: SGXUED. ISSN: 1000-3061.

AB The plasmid pXZ10145 has been isolated from 1 strain of *C. glutamicum* 1014. The plasmid confers chloramphenicol resistance and is 5.3kb, as determined by gel electrophoresis. A restriction endonuclease map of the plasmid was determined with BamHI, PstI, EcoRI, SalI, and double digested with BamHI + SalI, BamHI + PstI, PstI + SalI, EcoRI + PstI, and EcoRI + BamHI. One composite plasmid pXZ234 was constructed from plasmids pXZ10145 and pBR322 by recombination through their single BamHI sites. This plasmid was capable of replication in *Escherichia coli*, expressed ampicillin resistance in *E. coli*, and conferred chloramphenicol resistance.

L9 ANSWER 14 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1987:171700 Document No. 106:171700 Isolation and purification of CeqI endonuclease, an isoschizomer of EcoRV. Duda, E. G.; Izsvak, Z.; Orosz, A. (Inst. Biochem., Hung. Acad. Sci., Szeged, 6701, Hung.). Nucleic Acids Research, 15(3), 1334 (English) 1987. CODEN: NARHAD. ISSN: 0305-1048.

AB The EcoRV isoschizomer restriction endonuclease CeqI (I) was partially purified from *Corynebacterium equi* by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> fractionation and DEAE-cellulose chromatog. I, which was suitable for cloning, was further purified on aminopentyl- or aminohexyl-Sepharose or fast-protein liquid chromatog. Mono Q columns. I requires high salt (100-200 mM) for optimum activity and protection from heat inactivation. The cleavage specificity (GAT↓ATC/CTA↓TA G) (↓ indicates cleavage site) was the same as with EcoRV. I has a mol. weight of 300,000 and is dissociated to a 40,000-dalton, inactive form by >1M salt. Thus, the active enzyme is present as an aggregate structure.

L9 ANSWER 15 OF 15 CAPLUS COPYRIGHT 2005 ACS on STN

1984:624009 Document No. 101:224009 Plasmid pCC1. (Ajinomoto Co., Inc., Japan). Jpn. Kokai Tokkyo Koho JP 59143591 A2 19840817 Showa, 4 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 1983-17271 19830204.

AB Plasmid pCC1, which contains 4.2 kilobase pairs and 3 BglI, 2 AvaII, 1 BalI, 1 SmaI, 1 KpnI, and 1 HindIII restriction endonuclease sites, is prepared from glutamate-producing *Corynebacterium* species, especially *C. callunae*. Thus, *C. callunae* NRRL B-2244 strain was lysed with lysozyme and SDS and centrifuged; the supernatant fraction was treated with polyethylene glycol. The precipitate was resuspended in the conventional TEN buffer containing Tris-HCl, EDTA, and NaCl and subjected to CsCl-ethidium bromide d. gradient centrifugation to recover pCC1. Plasmid pCC1 is a cloning vector plasmid which uses coryneform bacteria as hosts.

=> E MORGAN R/AU

=> S E3,E7,E8,E75,E69,E80

77 "MORGAN R"/AU

8 "MORGAN R D"/AU

1 "MORGAN R DONALD"/AU

44 "MORGAN RICHARD"/AU  
 2 "MORGAN RHEA V"/AU  
 1 "MORGAN RICHARD DAVID"/AU  
 L10 133 ("MORGAN R"/AU OR "MORGAN R D"/AU OR "MORGAN R DONALD"/AU OR  
 "MORGAN RICHARD"/AU OR "MORGAN RHEA V"/AU OR "MORGAN RICHARD  
 DAVID"/AU)

=> E WALSH P/AU

=> S E3,E45

31 "WALSH P"/AU  
 2 "WALSH PAUL"/AU  
 L11 33 ("WALSH P"/AU OR "WALSH PAUL"/AU)

=> S L10,L11

L12 166 (L10 OR L11)

=> S L12 AND L4

L13 8 L12 AND L4

=> S L13 NOT L8

L14 8 L13 NOT L8

=> D 1-8 CBIB ABS

L14 ANSWER 1 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

2005:108494 Document No. 142:311988 Specificity Changes in the Evolution of  
 Type II Restriction Endonucleases: A biochemical and  
 bioinformatic analysis of restriction enzymes that  
 recognize unrelated sequences. Pingoud, Vera; Sudina, Anna; Geyer,  
 Hildegard; Bujnicki, Janusz M.; Lurz, Rudi; Lueder, Gerhild; Morgan,  
 Richard; Kubareva, Elena; Pingoud, Alfred (Institut fuer Biochemie,  
 Justus-Liebig-Universitaet, Giessen, D-35392, Germany). Journal of  
 Biological Chemistry, 280(6), 4289-4298 (English) 2005. CODEN: JBCHA3.  
 ISSN: 0021-9258. Publisher: American Society for Biochemistry and  
 Molecular Biology.

AB How restriction enzymes with their different specificities and mode of cleavage  
 evolved has been a long standing question in evolutionary biol. We have recently  
 shown that several Type II restriction endonucleases, namely SsoII (↓ CCNGG),  
 PspGI (↓ CCWGG), EcoRII (↓ CCWGG), NgoMIV (G ↓ CCGGC), and Cfr10I (R ↓ CCGGY),  
 which recognize similar DNA sequences (as indicated, where the downward arrows  
 denote cleavage position), share limited sequence similarity over an interrupted  
 stretch of .apprx.70 amino acid residues with MboI, a Type II restriction  
 endonuclease from Moraxella bovis (Pingoud, V., Conzelmann, C., Kinzebach, S.,  
 Sudina, A., Metelev, V., Kubareva, E., Bujnicki, J. M., Lurz, R., Luder, G., Xu,  
 S. Y., and Pingoud, A. (2003) J. Mol. Biol. 329, 913-929). Nevertheless, MboI  
 has a dissimilar DNA specificity (↓ GATC) compared with these enzymes. In this  
 study, we characterize MboI in detail to determine whether it utilizes a  
 mechanism of DNA recognition similar to SsoII, PspGI, EcoRII, NgoMIV, and Cfr10I.  
 Mutational analyses and photocross-linking expts. demonstrate that MboI exploits  
 the stretch of .apprx.70 amino acids for DNA recognition and cleavage. It is  
 therefore likely that MboI shares a common evolutionary origin with SsoII, PspGI,  
 EcoRII, NgoMIV, and Cfr10I. This is the first example of a relatively close  
 evolutionary link between Type II restriction enzymes of widely different  
 specificities.

L14 ANSWER 2 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

2002:666668 Document No. 138:33975 Functional analysis of iceA1, a  
 CATG-recognizing restriction endonuclease gene in  
 Helicobacter pylori. Xu, Qing; Morgan, R. D.; Roberts, R. J.;  
 Xu, S. Y.; van Doorn, L. J.; Donahue, J. P.; Miller, G. G.; Blaser, Martin  
 J. (Department of Microbiology and Immunology, Vanderbilt University  
 School of Medicine, Nashville, TN, 37232, USA). Nucleic Acids Research,

30(17), 3839-3847 (English) 2002. CODEN: NARHAD. ISSN: 0305-1048.  
Publisher: Oxford University Press.

- AB IceA1 in *Helicobacter pylori* is a homolog of NlaIII, which encodes the CATG-specific restriction endonuclease NlaIII in *Neisseria lactamica*. Anal. of iceA1 sequences from 49 *H. pylori* strains shows that a full-length NlaIII-like ORF is present in 10 strains, including CH4, but in other strains, including strain 60190, the ORFs are truncated due to a variety of mutations. Our goal was to determine whether iceA1 can encode a NlaIII-like endonuclease. In *Escherichia coli*, overexpression of iceA1 from CH4, but not from 60190, yielded NlaIII-like activity, indicating that the full-length iceA1 is a functional endonuclease gene. Repair of the iceA1 frameshift mutation in strain 60190 and its expression in *E. coli* yielded functional NlaIII-like activity. We conclude that iceA1 in CH4 is a functional restriction endonuclease gene, while iceA1 in 60190 is not, due to a frameshift mutation, but that its repair restores its restriction endonuclease activity.

L14 ANSWER 3 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1998:663441 Document No. 130:62752 Characterization of an extremely thermostable restriction enzyme, PspGI, from a *Pyrococcus* strain and cloning of the PspGI restriction-modification system in *Escherichia coli*. Morgan, Richard; Xiao, Jian-Ping; Xu, Shuang-Yong (New England Biolabs, Inc., Beverly, MA, 01915, USA). *Applied and Environmental Microbiology*, 64(10), 3669-3673 (English) 1998. CODEN: AEMIDF. ISSN: 0099-2240. Publisher: American Society for Microbiology.

- AB An extremely thermostable restriction endonuclease, PspGI, was purified from *Pyrococcus* sp. strain GI-H. PspGI is an isoschizomer of EcoRII and cleaves DNA before the first C in the sequence 5' .cxa.CCWGG 3' (W is A or T). PspGI digestion can be carried out at 65 to 85°. To express PspGI at high levels, the PspGI restriction-modification genes (pspGIR and pspGIM) were cloned in *Escherichia coli*. M.PspGI contains the conserved sequence motifs of  $\alpha$ -aminomethyltransferases; therefore, it must be an N4-cytosine methylase. M.PspGI shows 53% similarity to (44% identity with) its isoschizomer, M.MvaI from *Micrococcus variabilis*. In a segment of 87 amino acid residues, PspGI shows significant sequence similarity to EcoRII and to regions of SsoII and StyD4I which have a closely related recognition sequence (5' .cxa.CCNGG 3'). PspGI was expressed in *E. coli* via a T7 expression system. Recombinant PspGI was purified to near homogeneity and had a half-life of 2 h at 95°. PspGI remained active following 30 cycles of thermocycling; thus, it can be used in DNA-based diagnostic applications.

L14 ANSWER 4 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1994:675266 Document No. 121:275266 Molecular cloning of gene for NotI modification methylase and restriction endonuclease genes of *Nocardia otitidis-caviarum*. Morgan, Richard David; Benner, Jack Stanley; Claus, Toby Elizabeth (New England Biolabs, Inc., USA). *Eur. Pat. Appl. EP 607005 A2* 19940720, 21 pp. DESIGNATED STATES: R: DE, FR, GB. (English). CODEN: EPXXDW. APPLICATION: EP 1994-300162 19940111. PRIORITY: US 1993-3254 19930111.

- AB The 42-kDa NotI restriction endonuclease of *Nocardia otitidis-caviarum* was purified and partially degraded. The N-terminal amino acids of each peptide were determined and used for developing primers for cloning the gene. A partial DNA sequence of the gene was demonstrated.

L14 ANSWER 5 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1993:119896 Document No. 118:119896 A novel type II restriction endonuclease PmeI of *Pseudomonas mendocina* and its preparation. Morgan, Richard; Zhou, Bing (New England Biolabs, Inc., USA). *Eur. Pat. Appl. EP 517111 A1* 19921209, 10 pp. DESIGNATED STATES: R: DE, FR, GB. (English). CODEN: EPXXDW. APPLICATION: EP 1992-109085 19920529. PRIORITY: US 1991-710040 19910603.



AB A novel type II restriction endonuclease PmeI is separated from *Pseudomonas mendocina*. The enzyme having a defined cleavage site cleaves the double-stranded DNA of  $\lambda$  cl857 in 2 positions and adenovirus type 2 in 1 position. Incubation of the enzyme at 65° for 20 min inactivates its activity.

L14 ANSWER 6 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1992:629111 Document No. 117:229111 Purification of a novel type II restriction endonuclease from *Arthrobacter*.

Morgan, Richard (New England Biolabs, Inc., USA). Eur. Pat. Appl.

EP 498404 A1 19920812, 9 pp. DESIGNATED STATES: R: DE, FR, GB.

(English). CODEN: EPXXDW. APPLICATION: EP 1992-101929 19920205.

PRIORITY: US 1991-650802 19910205.

AB A type II restriction enzyme (AseI) with the cleavage site GG↓CGCGCC/CCGCGC↑GG is obtained from *Arthrobacter* NEB688. The enzyme was purified from cell lysates by ion-exchange chromatog. on DEAE-cellulose, heparin-Sepharose, and phosphocellulose in 36% yield and free of contaminating nucleases. Maximum activity was in a buffer containing KAc 50, Tris-acetate 20, Mg(Ac)2 10, dithiothreitol 1 mM (pH 7.9) at 25°.

L14 ANSWER 7 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1990:192673 Document No. 112:192673 Cloning and expression of the genes for AseI DNA methylase and restriction endonuclease in

*Escherichia coli*. Morgan, Richard (New England Biolabs, Inc.,

USA). Eur. Pat. Appl. EP 332406 A2 19890913, 9 pp. DESIGNATED STATES: R:

AT, BE, CH, DE, ES, FR, GB, GR, IT, LI, LU, NL, SE. (English). CODEN:

EPXXDW. APPLICATION: EP 1989-302277 19890307. PRIORITY: US 1988-164509

19880307.

AB The genes for the AseI restriction and modification enzymes of *Aquaspirillum serpens* (recognition sites: ATTAAT) are cloned and expressed in *Escherichia coli*. A HinPI digest of *A. serpens* DNA was cloned into the AccI site of pUC19 and a primary gene bank made in *E. coli*. A bulk plasmid DNA pepen. from this bank was digested with AseI and the digest transformed into *E. coli*. Transformants were resistant to AseI digestion because of the expression of the methylase gene. These transformants (14) were then analyzed by AseI and HinPI digestion of the plasmids. Five of these were AseI-resistant and showed the same HinPI digestion pattern. Assay of exts. of these transformants for restriction endonuclease activity showed the most productive transformant producing 105 units AseI/g cells.

L14 ANSWER 8 OF 8 CAPLUS COPYRIGHT 2005 ACS on STN

1987:550174 Document No. 107:150174 The cleavage site for the restriction endonucleases BanI and HgiC I is 5'

...G↓GPyPuCC...3'. Schildkraut, Ira; Lynch, James; Morgan,

Richard (New England Biolabs, Inc., Beverly, MA, 01915, USA).

Nucleic Acids Research, 15(13), 5492 (English) 1987. CODEN: NARHAD.

ISSN: 0305-1048.

AB Both restriction endonucleases BanI and HgiCI cleave the nucleotide sequence 5'...G↓GPyPuCC...3' sym. to leave a 4-base 5' extension, in contrast to the work of M. Kroger, et al. (1984), where the cleavage for HgiCI was reported as 5'...↓GGPyPuCC...3'.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	56352	restriction adj (enzyme or endonuclease)	US-PGPUB; USPAT	ADJ	ON	2005/09/30 10:57
L2	2	cstm i or (cstm adj (1 or i))	US-PGPUB; USPAT	ADJ	ON	2005/09/30 10:59
L3	12042	corynebacterium	US-PGPUB; USPAT	ADJ	ON	2005/09/30 10:58
L4	6128	l1 and l3	US-PGPUB; USPAT	ADJ	ON	2005/09/30 10:59
L5	0	l1 near l3	US-PGPUB; USPAT	ADJ	ON	2005/09/30 11:00
L6	255	l1 same l3	US-PGPUB; USPAT	ADJ	ON	2005/09/30 11:00
L7	5	l1 adj6 l3	US-PGPUB; USPAT	ADJ	ON	2005/09/30 11:02